EFFECT OF TEXTURE, MATURITY AND ENSILING OF MAIZE GRAIN ON RUMINAL PROTEIN DEGRADABILITY

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INTRODUCTION

In ruminants, protein is extensively degraded in the rumen. In modern protein systems it is taken into account that only dietary protein, which escapes degradation in the rumen and is digested postruminally, can be utilized by the ruminant animals.

Maize hybrids can vary widely in the ruminal degradability of grain (Flachowsky et al., 1992). Differences among hybrids were attributed to grain texture, i.e. dent or flint (Verbič et al., 1995). Philippeau and Michalet-Doreau (1997) reported that differences in grain degradation between dent and flint type hybrids were due to differences in degradation of starch as a main component of maize grain. Variability among hybrids was also found in degradation of protein (Verbič and Babnik, 1998; Philippeau et al., 1999). Other factors which affect *in sacco* degradation of maize grain in the rumen are maturity (Philippeau and Michalet-Doreau, 1997; Bal et al. 2000; Ettle et al., 2001), conservation method (Philippeau and Michalet-Doreau, 1998), fertilization with N (Babnik et al., 2002) and sample preparation before the incubation in the rumen (Philippeau and Michalet-Doreau, 1997; Philippeau and Michalet-Doreau, 1998; Matthé et al., 1999).

The objectives of our study were to examine the effects of genotype, maturity and ensiling of maize grain on the *in sacco* degradability of protein in the rumen. We also wanted to examine any possible interactions between the main effects which were investigated. With the aim of minimizing the effect of physical form and particle size on degradation characteristics, measurements were taken on undried samples which were ground in a manner which ensures similar particle size distribution irrespective of genotype, maturity or conservation method.

MATERIAL AND METHODS

Four commercial maize hybrids, two of them belonged to flint (Flint 1, Flint 2) and the other two to dent (Dent 1, Dent 2) type, were sown at the experimental fields Jablje (Latitude 46°08' N; Longitude 14°34' E; Altitude 305 m) on 6 May 1997 at a population density of 81,600 plants/ha. Maize was fertilized with 255 kg N/ha, 90 kg P_2O_5/ha and 90 kg K_2O/ha.

Ears were harvested manually at hard dough (1 Sept.), one-third milkline (10 Sept.) and two-thirds milkline (19 Sept.) stages of maturity and stored at –20º C. Kernels were removed from frozen ears and ground in a laboratory hammer mill without screen. Ground grain samples were then divided into three portions. One portion was stored at –20º C until the rumen study (Fresh), one portion was ensiled (Silage) and one portion was dried at 60º C (Dry). Silages were prepared in duplicates in glass laboratory silos with a volume of 1 l. Each of silos contained about 700 g of fresh material. Silage additive Plantanaze (Krka Novo mesto) at a theoretical rate of 1.0×10^5 cfu of lactic acid bacteria (*L. Plantarum* and *S. Faecium*) per g of fresh forage was added to ensure lactic acid fermentation in laboratory conditions. Silos were opened after 85 days.

In situ measurements of ruminal protein degradability were done using two ruminally cannulated nonlactating cows. They were offered a diet of hay (5 kg) and concentrate (2.5 kg) in two equal meals at 06.00 and 18.00. Degradability was determined as described by Ørskov et al. (1980). In order to remove microbial matter from undigested material, a modified frozen-rethawing technique according to Kamel et al. (1995) was used. Washing losses of protein (A) were determined by soaking bags with samples for 1 hour in hot water (39 ºC) instead of incubating them in the rumen and then washed as described previously.

Data of protein loss from the bags (p) at different incubation times (t) were fitted to the exponential equation $p=a+b(1-e^{-ct})$ as suggested by Ørskov and McDonald (1979). Coefficient a of exponential equation represents rapidly degradable fraction, coefficient b slowly degradable fraction and coefficient c degradation rate of fraction b. At first stage, losses at 3, 6, 12, 24 and 48 h incubations were taken into account in calculations of coefficients a, b and c. It was found that estimated rapidly degradable fraction of protein (a) deviated, on the average, from washing loss (A) only for 0.7%. This indicates that in maize grain there is no lag time in degradation of protein. Therefore, it was decided to include the washing loss into the calculations of coefficients a, b and c as a zero time incubation. Degradability of protein (DG) was calculated according to Ørskov and McDonald (1979) using the equation $DG = a+bc/(c+k)$. For k, a theoretical value of 0.05 h^{-1} was used.

Crude protein (CP; N×6.25) was analyzed according to the Kjeldahl method, and true protein according to method of Barnstein as described by Naumann and Bassler (1976).

Data were analyzed by least squares analysis of variance using General Linear Model procedure (STATGRAPHICS PLUS, 1994).

RESULTS AND DISCUSSION

Chemical Composition of Maize Grain

Chemical composition of maize grain is given in Table 1. DM concentration of the grain was significantly affected by the grain texture and harvest date. With advancing maturity DM concentration increased from 52.5 to 63.4% and from 43.9 to 59.7% in flint and dent type hybrids respectively. Although all the hybrids belonged to the same maturity class, grain of flint type hybrids was characterized by higher DM concentration than grain from dent hybrids (58.4 vs. 52.6%). However, as a result of lower DM concentration in the stover of flint maize, differences in whole plant DM between flint and dent hybrids were small (32.9 vs. 31.3%). DM concentrations of whole plant varied on average from 28.7% at first harvest date to 35.7 % at last harvest date. The results indicate that plants were harvested at maturity suitable for ensiling.

Table 1. Influence of grain texture and harvest date on chemical composition of maize grain

	DM concentration		True protein[1]		Crude protein
	Whole plant	Grain	Fresh	Silage	
	(%)				
Grain texture					
Flint	32.9[a]	58.4[b]	94.2[a]	70.1[a]	9.84[b]
Dent	31.3[a]	52.6[a]	94.6[a]	71.1[a]	9.26[a]
SEM	0.69	0.91	0.74	0.82	0.16
Harvest date					
1 Sept.	28.7[a]	48.2[a]	94.1[a]	71.9[a]	9.76[a]
10 Sept.	31.9[b]	56.8[b]	94.4[a]	70.5[a]	9.44[a]
19 Sept.	35.7[c]	61.6[c]	94.6[a]	69.5[a]	9.45[a]
SEM	0.86	1.11	0.91	1.01	0.19
			Significance (P)		
Grain texture (T)	NS[2]	< 0.01	NS	NS	< 0.01
Harvest date (D)	< 0.001	< 0.001	NS	NS	NS
T × D	NS	NS	NS	NS	NS

[a,b,c] Means with different superscripts within main factors differ significantly ($P < 0.05$)

[1] Expressed as % of crude protein

[2] $P \geq 0.05$

DM = Dry matter

Grain of flint maize contained more crude protein than grain of dent maize (9.84 vs. 9.26%, $P < 0.01$). Concentration of protein was not affected by the harvest date. True protein in maize grain represented on average 94.4% of crude protein and was affected neither by the texture nor by the maturity stage (Table 1).

No differences in the concentration of protein among unensiled and ensiled grain were observed. However, the proportion of true protein decreased on average from 94.4 to 70.6% during the ensiling process. Results are in accordance with the results of Baron et al. (1986) who found that soluble nonprotein N increased substantially during anaerobic fermentation of maize grain.

Factors Affecting Degradation of Protein in the Rumen

The effect of drying. Protein degradability of fresh and oven dried samples was compared only for two hybrids (one dent and one flint type) at two-thirds milkline maturity stage (Table 2). The drying process greatly affected protein degradability in the rumen. The results of this study confirm the findings of Matthe et al. (1999) saying that oven dried maize grain is degraded at a lower level compared to fresh grain. Therefore, it was decided that fresh samples would be used for the study of factors affecting protein degradability in the rumen.

Table 2. The effect of drying of crushed maize grain of two hybrids at two-thirds milkline maturity stage on degradation characteristics of protein in the rumen

	Degradation characteristics of protein			
	a	b	c	DG
	—— (%) ——		(/h)	(%)
Conservation				
Fresh	21.0^b	73.2^a	0.1209^b	72.3^b
Dry	18.3^a	76.6^b	0.0815^a	65.8^a
SEM	0.55	0.46	0.003	0.59
	Significance (P)			
Conservation (C)	< 0.05	< 0.01	< 0.001	< 0.01
Grain texture (T)	< 0.01	< 0.01	< 0.01	< 0.05
C × T	NS[1]	< 0.05	< 0.01	< 0.05

a = Rapidly degradable protein fraction, b = Slowly degradable protein fraction, c = Degradation rate of slowly degradable protein fraction, DG = Protein degradability.
[1] $P \geq 0.05$.

The effect of grain texture. Protein degradability varied widely between the hybrids of different grain texture ($P < 0.001$, Table 3). Significant variation was also observed among hybrids which were classified in the same texture class ($P < 0.001$). In comparison to grain of dent type hybrids, grain of flint type hybrids was characterized by lower protein degradability (77.3 vs. 68.8%) in the rumen. Higher protein degradability in dent type grain in comparison to flint was a consequence of higher rapidly degradable fraction of protein (25.0 vs. 20.5%), as well as a consequence of higher degradation rate of slowly degradable protein (0.1528 vs. 0.0951 h^{-1}). The results are in agreement with the results of Verbič and Babnik (1998) who reported higher protein degradability for whole plant maize silage made from dent type hybrid in comparison to silage, which was made from flint type hybrid.

Table 3. The effect of conservation, grain texture and harvest date on the rate and extent of ruminal protein degradation of maize grain

	Degradation characteristics of protein			
	a	b	c	DG
	(%)	(%)	(/h)	(%)
Conservation				
Fresh	23.0[a]	72.1[b]	0.1229[a]	73.0[a]
Silage	59.8[b]	38.0[a]	0.1377[b]	87.3[b]
SEM	0.17	0.22	0.0031	0.18
Grain texture				
Flint	38.5[a]	57.6[b]	0.1080[a]	77.0[a]
Dent	44.3[b]	52.5[a]	0.1525[b]	83.3[b]
SEM	0.17	0.22	0.0031	0.18
Harvest date				
1 Sept.	44.5[b]	52.5[a]	0.1414[b]	82.0[c]
10 Sept.	40.0[a]	56.1[b]	0.1301[ab]	79.6[b]
19 Sept.	39.7[a]	56.5[b]	0.1193[a]	78.9[a]
SEM	0.21	0.28	0.0038	0.22
Hybrid (Grain texture)				
Flint 1	37.8[a]	58.1[c]	0.1048[a]	76.6[a]
Flint 2	39.2[b]	57.0[b]	0.1113[a]	77.4[b]
Dent 1	47.9[d]	48.6[a]	0.1716[c]	85.5[d]
Dent 2	40.7[c]	56.4[b]	0.1334[b]	81.2[c]
SEM	0.24	0.32	0.0044	0.25
Conservation × Grain texture				
Fresh × Flint	20.5[a]	73.8[d]	0.0951[a]	68.8[a]
Silage × Flint	55.1[c]	42.4[b]	0.1145[b]	84.4[c]
Fresh × Dent	25.0[b]	70.5[c]	0.1528[c]	77.3[b]
Silage × Dent	63.6[d]	34.4[a]	0.1522[c]	89.4[d]
SEM	0.25	0.32	0.0044	0.25
Conservation × Harvest date				
Fresh × 1 Sept.	26.4[c]	69.6[d]	0.1281[ab]	74.8[b]
Fresh × 10 Sept.	22.2[b]	72.2[e]	0.1261[ab]	72.6[a]
Fresh × 19 Sept.	20.4[a]	74.4[f]	0.1144[a]	71.7[a]
Silage × 1 Sept.	62.6[f]	35.4[a]	0.1546[c]	89.2[d]
Silage × 10 Sept.	57.8[d]	40.1[c]	0.1342[b]	86.6[c]
Silage × 19 Sept.	59.1[e]	38.6[b]	0.1242[ab]	86.1[c]
SEM	0.30	0.39	0.0054	0.31
Grain texture × Harvest date				
Flint × 1 Sept.	39.1[b]	57.1[c]	0.1207[a]	78.7[c]
Flint × 10 Sept.	36.2[a]	59.7[d]	0.0946[b]	74.9[a]
Flint × 19 Sept.	38.1[b]	57.6[c]	0.0991[ab]	76.3[b]
Dent × 1 Sept.	48.3[d]	49.0[a]	0.1553[cd]	84.6[e]
Dent × 10 Sept.	42.8[c]	53.8[b]	0.1624[c]	83.7[e]
Dent × 19 Sept.	41.8[c]	54.7[b]	0.1398[ad]	81.7[d]
SEM	0.30	0.39	0.0054	0.31
	Significance (P)			
Conservation (C)	< 0.001	< 0.001	< 0.01	< 0.001
Grain texture (T)	< 0.001	< 0.001	< 0.001	< 0.001
Harvest date (D)	< 0.001	< 0.001	< 0.01	< 0.001
Hybrid (Grain texture); H(T)	< 0.001	< 0.001	< 0.001	< 0.001
C × T	< 0.001	< 0.001	< 0.01	< 0.001
C × D	< 0.001	< 0.001	NS	NS
C × H(T)	< 0.001	< 0.001	< 0.001	< 0.01
T × D	< 0.001	< 0.001	< 0.01	< 0.001
D × H(T)	< 0.001	< 0.001	< 0.05	< 0.001
C × G × D	NS[1]	NS	< 0.01	< 0.01
C × D × H(T)	< 0.01	NS	< 0.01	< 0.01

[1] $P \geq 0.05$.

[a,b,c]Means within main factors and their interactions with a common superscript do not differ significantly ($P > 0.05$).

All terms are defined in Table 2.

The effect of maturity. The results of the present study have indicated that protein degradability of fresh maize grain at hard dough stage (1 Sept.) was significantly higher than at one-third milkline (10 Sept.) and two-thirds milkline (19 Sept.) stages (74.8 vs. 72.6 and 71.7%, Table 3). A consistent decrease in protein degradability in response to advanced maturity was observed only in dent maize. In flint maize protein degradability from first harvest date to second harvest date slightly decreased and thereafter, degradability was increased again. However, we have no explanation for it. The effect of maturity was considerably less important factor affecting ruminal protein degradability than the grain texture.

The effect of ensiling. The results of this study confirm that large changes in degradability of protein in the rumen occur during the ensiling process (Table 3). Relative to fresh grain protein degradability of ensiled grain increased from 73.0 to 87.3%. Ensiling process increased rapidly degradable protein (a). Slowly degradable protein (b) decreased during the ensiling, while their degradation rate (c) increased (Table 3).

To our knowledge, there are no data in the literature on the effect of ensiling of maize grain on degradation of protein in the rumen. The results of the current study are in agreement with the studies on grassland forages which suggested that the protein degradability during the ensiling process increased (Lopez et al., 1991, Petit and Tremblay, 1992). Increase in protein degradability during the ensiling process was accompanied by a pronounced decline in true protein fraction (Table 1).

Significant interaction between grain texture and conservation (Table 3) indicates that changes in protein degradability during the ensiling depend on genotype. The increase in protein degradability during the ensiling process was larger in flint type hybrids (15.6 and 11.7 percentage units) than in dent type hybrids (12.1 and 10.2 percentage units).

CONCLUSION

The results of current study indicate that grain texture and ensiling markedly affected protein degradation of maize grain in the rumen. The effect of maturity was less pronounced. Protein degradability was higher in dent type grain than in flint grain. Relative to fresh samples protein degradability of ensiled samples was considerably higher. The increase in protein degradability during the ensiling process was higher in flint type grain than in dent.

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